EXHIBIT

8

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05-CV-0329 GKF-PJC

INDICATORS FOR WATERBORNE PATHOGENS

Committee on Indicators for Waterborne Pathogens

Board on Life Sciences

Water Science and Technology Board

Division on Earth and Life Studies

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COMMITTEE ON INDICATORS FOR WATERBORNE PATHOGENS[1]

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RICARDO DELEON, Metropolitan Water District of Southern California, La Verne
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DEBORAH A. LEVY, Centers for Disease Control and Prevention, Atlanta, Georgia
J. VAUN MCARTHUR, University of Georgia, Savannah River Ecology Laboratory, Aiken, South Carolina
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DAVID R. WALT, Tufts University, Boston, Massachusetts
STEPHEN B. WEISBERG, Southern California Coastal Water Research Project Authority, Westminster
MARYLYNN V. YATES, University of California, Riverside

Staff

MARK C. GIBSON, Study Director
JENNIFER KUZMA, Co-Study Director *(until January 2003)*
SETH H. STRONGIN, Project Assistant

[1]The activities of the Committee on Indicators for Waterborne Pathogens were organized and supported by the National Research Council's Board on Life Sciences (lead) and Water Science and Technology Board. Biographical sketches of committee members are contained in Appendix E and current rosters of the two parent boards are contained in Appendix D.

**WORKSHOP ON INDICATORS FOR
WATERBORNE PATHOGENS PARTICIPANTS**

September 4, 2002

JOHN M. COLFORD, JR., University of California, Berkeley
CHRISTOPHER S. CROCKETT, Philadelphia Water Department
RAYMOND P. MARIELLA, Lawrence Livermore National Laboratory
PETER MARSDEN, Drinking Water Inspectorate of the United Kingdom
J. MICHAEL RAMSEY, Oak Ridge National Laboratory
GEOFFREY I. SCOTT, National Oceanic and Atmospheric Administration
MARK D. SOBSEY, University of North Carolina, Chapel Hill
TIMOTHY J. WADE, University of California, Berkeley

Preface

The use of bacterial indicator organisms to signal the possible presence of human pathogens in drinking water began more than a century ago in the United States, at a time when contamination of drinking and source waters by enteric bacterial pathogens, such as the typhoid bacillus, was a major public health threat. In subsequent decades, the use of bacterial indicators, predominantly coliforms, has been expanded to U.S. ambient, recreational, and shellfish waters and continues to focus on identification of fecal contamination, principally of human origin. Although these approaches have been extremely effective in reducing outbreaks of waterborne human disease, significant numbers of such outbreaks are still reported annually, many of unknown etiologic origin, and it is generally agreed that a substantial number of occurrences of waterborne human disease go unrecognized or unreported.

Recent advances in understanding the diversity and ecology of waterborne human pathogens as well as the ongoing rapid development of new techniques for detection and identification of waterborne microbes make it timely to reevaluate the standard indicators and indicator approaches employed to determine the microbiological quality of waters used for recreation or as sources of drinking water. Accordingly, the National Research Council (NRC) formed the Committee on Indicators for Waterborne Pathogens in 2002 at the request of the U.S. Environmental Protection Agency (EPA) to report on candidate indicators and/or indicator approaches (including technologies for detection) for assessing contamination of U.S. recreational waters and source water (including groundwater) for drinking water. The original charge to the committee excluded coastal marine and marine-estuarine waters, but these were added after subsequent discussion with EPA, and it was agreed that the study would then give less emphasis to some other aspects of the charge as described in Chapter 1. For example, the committee did not explicitly address indicators of water treatment performance. Furthermore, the report does not specifically address the threat of bioterrorism or the protection of vulnerable subpopulations such as infants and immunocompromised persons regarding microbial water quality.

To address its charge, the Committee on Indicators for Waterborne Pathogens met four times, starting in April 2002. The committee quickly concluded that it is not possible to identify a single, unique indicator or even a small set of indicators that is capable of identifying all classes of waterborne pathogens of public health concern for all applications and water media. Rather, priority should be given to the development of a phased monitoring approach for assessing microbial water quality that relies on a flexible "tool box" containing a spectrum of indicators and indicator approaches (to include direct monitoring of pathogens) that can be matched according to specific circumstances and needs. Thus, the committee did not conduct a comprehensive evaluation of candidate indicators or specific pathogens per se.

The committee would like to thank the many experts who contributed to this report by participating and/or speaking at committee meetings, including Rita Schoeny, Betsy Southerland, Ephraim King, Alfred Dufour, and Rebecca Calderon, EPA; and Roger Fujioka, University of Hawaii.

The committee also sponsored a one-day public workshop on candidate indicators and indicator approaches for waterborne pathogens on September 4, 2002, in Washington, D.C. This workshop provided insight on a wide variety of subjects related to the committee's charge, ranging from epidemiology to emerging detection technologies. The names and affiliations of the workshop presenters are listed in the front of this report.

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the NRC in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We thank the following individuals for their review of this report: Jennifer Clancy, Clancy Environmental Consultants, Inc.; James Crook, Consultant; Mark Gold, Heal the Bay; Robert Haselkorn, University of Chicago; Mark LeChevallier, American Water; Laura Leff, Kent State University; Daniel Lim, University of South Florida; Christine Moe, Emory University; Erik Olson, Natural Resources Defense Council; David Relman, Stanford University; and Gary Toranzos, University of Puerto Rico.

Although the reviewers above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by Edward Bouwer, Johns Hopkins University. Appointed by the NRC, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the NRC.

This report would not have been possible without the hard work and dedication of Mark Gibson, study director and staff officer for the NRC's Water Science and Technology Board. The committee would like to thank project assistant Seth Strongin from the Board on Life Sciences (BLS) for logistical support throughout the study. We would also like to thank former co-study director Jennifer Kuzma and research associate Laura Holliday of the BLS for their early contributions to this report.

Finally, I would like to thank the 12 members of this committee for bringing this report together. Their diverse backgrounds and perspectives provided for lively and insightful discussions throughout the course of the entire study.

Mary Jane Osborn, *Chair*

Contents

Executive Summary

BACKGROUND

The establishment of sanitary practices in the late nineteenth century for the disposal of sewage and the increasing use of filtration and chlorination of drinking water throughout the twentieth century resulted in a dramatic decrease in waterborne diseases such as cholera and typhoid fever in the United States. Despite these historical efforts, ongoing advances in water and wastewater treatment, and several layers of federal, state, and local government laws, regulations, and guidance designed to protect public water supplies from contamination, waterborne disease outbreaks still occur every year in the United States. Furthermore, epidemiologists generally agree that these documented outbreaks represent only a fraction of the total that actually occur because many go undetected or unreported.

In order to protect public health, and as mandated in the Clean Water Act and the Safe Drinking Water Act (SDWA), it is important to have accurate, reliable, and scientifically defensible methods for determining whether source waters for drinking water and recreational waters are contaminated by pathogens and to what extent. For more than 100 years, U.S. public health personnel have relied extensively on an indicator organism approach to assess the microbiological quality of drinking water. More specifically, these enteric bacterial indicator microorganisms (predominantly "coliforms"[1]) are typically used to detect the possible

[1]Coliforms include several genera of bacteria belonging to the family Enterobacteriaceae, of which *Escherichia coli* is the most important member. The historical definition of this group is based on the method (lactose fermentation) used for its detection (see Chapter 1 for further information).

presence of microbial contamination of drinking water from human waste. The use of coliforms was later expanded and adopted for ambient, recreational, and shellfish waters and continues to focus on identification of fecal contamination.

Over the long history of their development and use, the current bacterial indicator approaches have become standardized, are relatively easy and inexpensive to use, and constitute a cornerstone of local, state, and federal monitoring and regulatory programs. An increased understanding of the diversity of waterborne pathogens, their sources, physiology, and ecology, however, has resulted in a growing understanding that the use of bacterial indicators may not be as universally protective as was once thought. For example, the superior environmental survival of pathogenic viruses and protozoa raised serious questions about the suitability of relying on relatively short-lived coliforms as an indicator of the microbiological quality of water. That is, while the presence of coliforms could still be taken as a sign of fecal contamination, the absence of coliforms could no longer be taken as assurance that the water was uncontaminated. Thus, existing bacterial indicators and indicator approaches do not in all circumstances identify all potential waterborne pathogens. Furthermore, recent and forecasted advances in microbiology, molecular biology, and analytical chemistry make it timely to reassess the current paradigm of relying predominantly or exclusively on traditional bacterial indicators for waterborne pathogens. Nonetheless, indicator approaches will still be required for the foreseeable future because it is not practical or feasible to monitor for the complete spectrum of microorganisms that may occur in source waters for drinking water and recreational waters, and many known pathogens are difficult to detect directly and reliably in water samples.

This report was written by the National Research Council (NRC) Committee on Indicators for Waterborne Pathogens—jointly overseen by the NRC's Board on Life Sciences and Water Science and Technology Board—and comprised of 12 volunteer experts in microbiology, waterborne pathogens (bacteriology, virology, parasitology), aquatic microbial ecology, microbial risk assessment, water quality standards and regulations, environmental engineering, biochemistry and molecular biology, detection methods, and epidemiology and public health. This report's contents, conclusions, and recommendations are based on a review of relevant technical literature, information gathered at four committee meetings, a public workshop on indicators for waterborne pathogens (held on September 4, 2002), and the collective expertise of committee members. Furthermore, because of space limitations, this Executive Summary includes only the major conclusions and related recommendations of the committee in the general order of their appearance in the report. More detailed conclusions and recommendations can be found within individual chapters and are summarized at the end of each chapter.

The committee was formed in early 2002 at the request of the U.S. Environmental Protection Agency (EPA) Office of Water and originally charged to report on candidate indicators and/or indicator approaches (including detection technologies) for microbial pathogen contamination in U.S. recreational waters

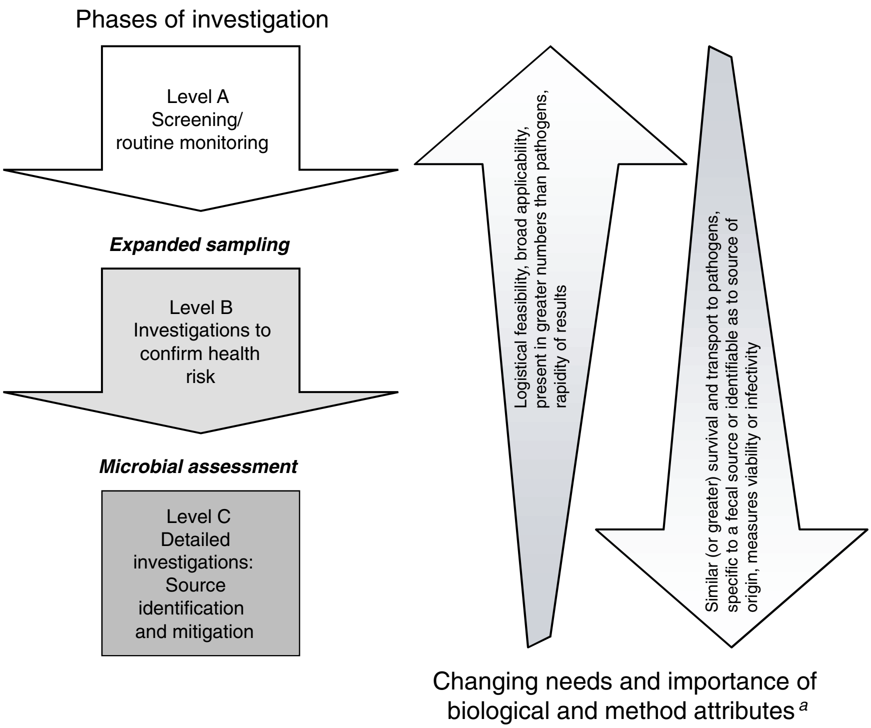

FIGURE ES-1 Recommended three-level phased monitoring framework for selection and use of indicators and indicator approaches for waterborne pathogens.
[a]Not all biological and method attributes summarized in Box ES-2 are included in this figure, nor are they listed in order of importance.

are not initiated on a routine basis, but would typically be undertaken when screening indicators persist at high levels without a clearly identifiable contamination source. Many of the new and emerging biological measurement methods and detection technologies described in Chapter 5 and Appendix C will be useful at this stage of investigation. Since confirmation studies focus on assessing health risk, the most important indicator biological attributes during this phase are correlation with contamination sources and transport or survival behavior similar to pathogens.

The third phase (Level C) involves studies to determine the sources of microbial contamination so that the health risk can be abated through a variety of engineering and policy solutions. However, this report focuses on the identification of sources of microbial contamination rather than their mitigation. In some cases, source identification is accomplished through expanded spatial sampling to look for gradients in indicator organisms or pathogens. Where recreational waters are for millennia. Bacteria and eukaryotes alike are then subject to new "harsh" environments. Interactions among species that normally do not occur have resulted in the panmixis of various genes and gene combinations (i.e., integrons and other transposable elements).

Other factors that must be considered regarding bacterial persistence in the environment, especially for bacteria from human or animal reservoirs, is the extent to which they are subjected to environmental stresses (such as extreme temperatures and pH levels, exposure to UV radiation in sunlight and toxic chemicals) that cause physiological stress and damage that is generally termed "injury." Injury can range in severity and the effects of such injury influence bacterial detection by culture and other methods, as well as bacterial infectivity for human or animal hosts.

Kurath and Morita (1983) called cells that could grow on media viable, but they recognized that most of the bacteria in their samples (>10 times the number of CFUs) had metabolic activity but did not grow on the culture plates. Bacteria that become injured by losing the ability to multiply (form colonies or grow in liquid media), but remain otherwise completely functional as individuals and metabolically active, have been termed "viable but non-culturable" (VBNC; Oliver, 1993). This condition may be due to nutrient deprivation or to the effects of a variety of environmental stresses (Roszak and Colwell, 1987). Many types of bacteria that are injured to varying extents and may be VBNC can be identified from samples using biochemical, immunological, and nucleic acid molecular techniques. Important unresolved questions about VBNC bacteria are what ecological role they play and whether or not they are infectious for human or animal hosts. In this regard, the mere presence of a bacterium, especially when detected by non-culture methods, does not necessarily imply ecological importance (Morita, 1997) or human health risk.

Several species of bacteria including frank human pathogens such as *Vibrio spp.*, *Escherichia coli*, *Campylobacter spp.*, *Salmonella spp.*, *Micrococcu*s, and *Pseudomonas* have been found to be VBNC under a variety of conditions. A general concern is that many other waterborne bacterial pathogen and indicator species will be found that express this trait of non-culturablity and that this condition may confound the reliability of various microbial indicators that are based on culture techniques. However, as VBNC cells are metabolically active, indicators that measure some correlate or product of metabolism might be developed that are capable of monitoring these targets even when these cells cannot be cultured. Therefore, detection of bacteria by non-culture methods is both possible and a potentially useful measure of the presence and concentrations of these types of bacteria (see Chapter 5).

It is important to note that the environmental and public health significance of injured bacteria, especially those that are VBNC, remains controversial and uncertain (Bogosian and Bourneuf, 2001). As indicated in Chapter 5, there is considerable evidence that VBNC bacteria are not infectious for human or animals as well as some evidence that they are. Because of such conflicting evidence and the uncertainties of their public health significance, VBNC bacteria are not addressed or discussed in detail in this report. However, there are good reasons to address the relationships between injured bacteria and their detection by various biochemical, immunological, and nucleic acid methods, and these are covered in Chapter 5 and Appendix C.

Dispersal

Although bacteria and other microbes are widely dispersed in nature, not all bacteria are found everywhere. Whether transported and imported bacteria are capable of survival under new or novel environmental conditions is not known. In freshwater lotic ecosystems, many bacteria in transport are allochthonous, having originated from neighboring terrestrial systems and washed into the aquatic system. Many of these bacteria are not actively growing and presumably contribute little to any ecosystem process (Edwards et al., 1990). Because of the possibility of waterborne pathogens surviving and replicating in various environmental reservoirs however, an understanding of mechanisms of dispersal is important.

Bacteria and other microbes that successfully replicate within a system can take advantage of dispersal mechanisms to both move longitudinally within a waterbody and escape a waterbody. Bacteria can also use dispersal vectors such as formation of aerosols, invection, organic foams, arthropods, and vertebrates either actively or passively.

Abiotic Mechanisms of Dispersal

Long-distance dispersal of waterborne pathogens and bacterial indicators is dependent on the movement of bacteria within waterways and whether they can exit and survive outside the waterbody. Regarding the latter, bacteria can effectively escape the aquatic environment in several ways.

Aerosol Formation The formation of aerosols is a function of the geology of a watercourse. Any turbulence caused by rocks, boulders, and woody structures that make water splash or cause wave action results in the formation of aerosols. Depending on the size of the droplets, the aerosols are transported to varying degrees into the atmosphere. The types of bacterial species found in aerosols should be proportional to those normally found and those transported in the water. Thus, aerosol formation below a sewage treatment plant outfall would be expected to have higher proportions of enteric bacteria than aerosols created either upstream or far downstream of an outfall. Very little research has been conducted in the last two decades on aerosol formation and bacterial transport resulting from sewage treatment practices (e.g., EPA, 1980). However, Rosas et al. (1993) sampled the air over sewage treatment plants and at various distances from the contamination from study to study, and may be one of the reasons for the differences in recreational water indicators and standards among states (see Tables 1-4 and 4-1).

The use of indicators is based on the presumption that they co-occur at a constant ratio with illness-causing pathogens. This premise is flawed because indicator levels in the gastrointestinal tract may vary within a narrow range, but pathogen concentration is highly variable and dependent on which pathogens are in the population at what levels at specific times. Furthermore, upon leaving the intestinal tract, microbial indicators and pathogens degrade at different rates that are mediated by factors such as their resistance to aerobic conditions, ultraviolet radiation, temperature changes, and salinity. As a result, the epidemiological relationship between indicator density and illness patterns can differ depending on the age of the source material, as well as local meteorological and other environmental conditions. Several studies also have found that some indicator bacteria can grow outside the human or animal intestinal system (Desmarais et al., 2002; Fujioka, 2001; Hardina and Fujioka, 1991; Solo-Gabrielle et al., 2000; see also Chapter 3), further confounding the correlation between pathogens and indicators.

The underlying epidemiologic studies are also limited because many reported failures of beach water quality standards are associated with nonpoint source contamination (Lipp et al., 2001a; Noble et al., 2000; Schiff et al., 2003), but the epidemiologic studies used to establish recreational bathing water standards (EPA, 1986) have been based primarily on exposure to human fecal-dominated point source contamination (Haile et al., 1999). Since nonpoint sources generally have a higher percentage of animal fecal contributions, and animals shed bacterial indicators without some of the accompanying human pathogens, there is considerable uncertainty in extrapolating present standards to nonpoint source situations. A poor correlation between bacterial indicators and virus concentrations has been found in the study of nonpoint sources and water quality (Jiang et al., 2001; Noble and Fuhrman, 2001). However, when a human source, such as septic systems, has been present, enterococci have been significantly correlated with viruses (Lipp et al., 2001a).

A major problem with present water contact warning systems is that bacterial indicator concentrations are spatially and temporally variable and most sampling is too infrequent to transcend this granularity.[1] Taggart (2002) found that sequential samples collected at the same location typically varied by a factor of two and samples 100 meters apart typically differed tenfold. Cheung et al. (1990) found

[1]For the purpose of this report, the term “granularity” refers to both the natural spatial and temporal variability of pathogens and indicator organisms that occur (and can be measured) in the environment and the level of coarseness or detail that is used in obtaining such measurements. As such, the term has a more specific meaning than “variability,” which is more commonly used throughout the report.